Comm.
Dr. Bing
Dr. Loosli
Dr. Sommers

CHRONIC PULMONARY DISEASES

#862R1
862 - 7/1/72 - 6/30/73

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☒ Second Renewal ☐

Date: Jan. 24, 1973

RECEIVED
JAN 29 1973

1. Principal Investigator (give title and degrees):

James Travis, Ph.D. Associate Professor

2. Institution & address:

Department of Biochemistry
University of Georgia
Athens, Georgia 30601

3. Department(s) where research will be done or collaboration provided:

Department of Biochemistry, College of Arts and Sciences

4. Short title of study:

Biochemistry of Chronic Obstructive Lung Disease

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims:

The preliminary results obtained indicate that more than one hydrolytic enzyme is probably responsible for the onset of this type of emphysema. Thus, we are now studying more than one enzyme in order to determine the means by which lung degradation occurs.

7. How results to date have changed earlier working hypothesis:

The hypothesis remains the same; that is, degradation of lung tissue by hydrolytic enzymes occurs because of the lack of alpha-1-antitrypsin in certain individuals.

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Source: https://www.industrydocuments.ucsf.edu/docs/nglf0000

8. Any additional facilities now required? Describe briefly:

The very heavy use of chromatographic equipment for the purification of both protease inhibitors from plasma as well as hydrolytic enzymes from leukocytic granules makes the purchase of another fraction collector and a UV monitor of the utmost necessity.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

Not Applicable

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

1. Properties of an Inactive Form of Human alpha-1-antitrypsin Purified by Affinity Chromatography. R. Pannell and J. Travis
   Federation Proceedings, 1973. (Abstract)

2. Human Pancreatic Enzymes: Isolation and Properties of a Second Form of Human Trypsin. P. Mallory and J. Travis
   Biochemistry (submitted for publication)

3. Human Pancreatic Enzymes: Purification and Properties of an Elastase-like Enzyme (Pseudoelastase). P. Mallory and J. Travis
   Biochemistry (submitted for publication)

10035393378

12. Summary progress report (append in standard form as separate document, unless recently submitted)

Source: https://www.industrydocuments.ucsf.edu/docs/nglp0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
James Travis, Associate Professor	40	-0-
Research Associate (to be named)	100	8000
Fringe Benefits (14%)		1120
Technical		
Joanne Betso	50	3500
Fringe Benefits (14%)		490
Sub-Total for A		13110
B. Consumable supplies (by major categories)		
Glassware		1500
Chemicals		1000
Biological Materials		500
Sub-Total for B		3000
C. Other expenses (itemize)		
Travel (Foreign-700; Domestic-200)		900
Publication Costs		500
Sub-Total for C		1400
Running Total of A + B + C		17510
D. Permanent equipment (itemize)		
Isco UV Monitor and Accessories		1500
Isco Fraction Collector		600
Sub-Total for D		2100
E. Indirect costs (15% of A+B+C)		2627
Total request		22237

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Source: https://www.industrydocuments.ucsf.edu/docs/ngpl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Proteolytic Enzymes of the Blood and Pancreas	NIH HL14778	22957	Jan 1/73 - Dec 31/73
Proteolytic Enzymes of the Blood and Pancreas	NIH Career Development Award 1K04 HL70264	20410	July 1/72 - June 30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name James Travis

Signature [signature] Date 1/23/73

Telephone 404 (Area Code) 542-1334 (Number) 25 (Extension)

Checks payable to

The University of Georgia

Mailing address for check:

Acting Vice President for Business and Finance

The University of Georgia

Athens, Georgia 30602

Responsible officer of institution

Typed Name Robert C. Anderson

Title Vice President for Research

Signature [signature] Date 1/24/73

Telephone 404 (Area Code) 542-3360 (Number) (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/ngdd0000

10. Experimental Protecol for Coming Year

During the coming year a detailed survey of all of the proteolytic enzymes present in granules will be made as well as the effects of both alpha-1-antitrypsin and alpha-2-macroglobulin on their activities. The continued isolation of the elastaese component(s) will be made and the properties of the enzyme(s) studied. It is difficult at this stage to attribute a great significance to leukocytic elastase(s) as the primary enzyme(s) responsible for lesions in the basement membrane of the alveolar wall. The amount of activity seems too low to account for this digestion and the fact that alpha-2-macroglobulin is still present in alpha-1-antitrypsin deficient patients would suggest that at least some of the elastase activity would be removed by this protein. It may well be that several enzymes are acting synergistically to cause the digestion of the alveolar membrane or that a proteolytic enzyme, not inhibitable by alpha-2-macroglobulin but inhibitable by alpha-1-antitrypsin, is responsible.

A shift to the isolation of alpha-1-antitrypsin by the techniques described in section 12 will be made, which should result in the isolation of at least the two most important forms of this protein, type MM and type ZZ. The comparative properties of each enzyme inhibitor will be studied to determine what differences might account for the low concentration of type ZZ alpha-1-antitrypsin in plasma.

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Source: https://www.industrydocuments.ucsf.edu/docs/gpl10000

12. Summary Progress Report

The objectives of this project were to isolate human leukocytic elastase as well as human alpha-1-antitrypsin and determine the relationship of these proteins to each other as well as their role in the onset of chronic obstructive lung disease. In the past six months since this project was initiated we have succeeded in purifying to homogeneity an inactive form of human alpha-1-antitrypsin which we intend to use to study some of the physical and chemical properties of this protein. Furthermore, this protein is also being used to produce antibodies which will be used for the preparation of the active form of the inhibitor by affinity chromatography More recently we have found that by using a sepharose-blue dextran derivative we can bing virtually all of the albumin in plasma to this material. Other plasma proteins bind as well but alpha-1-antitrypsin and alpha-2-macroglobulin pass directly through a column of this material without loss of activity. In fact, using plasma which has been passed through a column of Sephadex G-75 prior to passage through the sepharose-blue dextran column, we have been able to isolate fractions containg only two components, one of which is alpha-1-antitrypsin, and the other probably orosomucoid. we feel it should be a simple matter of using ion-exchange chromatography on DEAE-cellulose to isolate the active alpha-1-antitrypsin component in pure form. This technique should also be useful for the isolation of alpha-1-antitrypsin from the plasma of type ZZ individuals who have presented the symptoms of chronic obstructive lung disease. The removal of the albumin fraction will allow the concentration of large volumes of plasma fractions so that the alpha-1-antitrypsin (type ZZ) may be isolated. The technique should have even more far reaching implications for the isolation of trace proteins in plasma heretofore contaminated by the large quantities of albumin normally found in plasma.

The purification of leukocytic elastase to homogeneity on a large scale has been hampered by the strong binding of nucleic acids and hemoglobin to the enzyme, resulting in its inactivation. we have overcome these problems by a) working exclusively with leukocytic granules, b) fractionating the granule extract between 0-60% with $(NH_4)_2SO_4$, c) chromatographing on DEAE-cellulose, d) chromatographing on Sephadex G-75. This last step results in the separation of two proteolytic components both of which appear to be elastolytic in nature. However, neither component is homogenous at this stage.

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Source: https://www.industrydocuments.ucsf.edu/docs/qgpl0000